

I. IN THE CLAIMS (CLEAN SHEET)

1. A method of releasing a protein of interest from host cells comprising contacting the cells with a detergent and a reducing agent, wherein the active ingredient is an amphipathic charged amine or an amphipathic charged amine oxide.
2. The method of Claim 1, wherein the detergent is selected from the group consisting of:
Tributylphosphate, dimethyldecylamine, dimethyltridecylamine, dimethylundecylamine, dimethyldidecylamine, dimethyltetradecylamine, dimethylhexadecylamine, dimethyldecylamineoxide, dimethylundecylamineoxide, dimethyldidecylamineoxide, dimethyltetradecylamineoxide and dimethyltridecylamineoxide.
3. The method of claim 1, wherein the detergent is not dimethyltridecylamine.
4. The method of claim 1, further comprising adding glycerol to the suspended host cells.

5. The method of claim 4, wherein the detergent comprises a concentration of between about 0.01 to the solubility limit of the detergent.

A1 6. The method of claim 4, wherein the glycerol comprises a concentration of between about 0.6 to about 20 percent.

7. The method of claim 5, wherein glycerol comprises a concentration of between about 0.6 to about 6 percent.

8. The method of claim 1, wherein the reducing agent is selected from the group consisting of Dithiothreitol (DTT); Dithioerythritol (DTE); Cysteine (Cys) and Tris 2-carboxyethylphosphine (TCEP).

9. The method of claim 8, wherein the reducing agent is at a concentration of from about 0.1 mM to about 100 mM..

10. The method of Claim 1, wherein the host cells are *Pichia pastoris* cells.

A2 Rule 11.126 11-12. The method of claim 1, further comprising the step of incubating the solution from about 40 minutes to about 72 hours.

Rule
1.126
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13. The method of Claim ¹¹~~12~~, wherein the incubation is from about 90 minutes to about 24 hours.

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14. The method of Claim 1, further comprising the step of incubating the solution at a temperature of between about 2°C and about 50°C.

Rule
1.126
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15. The method of Claim 1 wherein the detergent is added to the cells at a concentration up to two hundred times the concentration of the solution.

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16. The method of Claim 1 wherein the pH of the solution ranges from about pH 5.0 to about pH 8.0.
